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Abstract

Soil soluble nitrogen (N) is crucial to the N nutrition and productivity of plants. Consequently, understanding the factors that affect its pool size and composition is of considerable importance. Here, six typical forest types in northeast China were investigated to determine the dynamics of soil soluble N across seasons and plant communities, and the potential drivers. Soil free amino acids, NH_4^+, NO_3^-, dissolved organic N (DON) and a variety of soil characteristics were measured over the growing season (from May to September). Seasonality showed a stronger effect on the availability of soil inorganic N and free amino acids than vegetation. The coefficients of variation of soil inorganic N, amino acid-N and the potential drivers (moisture and DON) appeared to be greater for season, and the concentrations of these available N sources tended to be higher at the beginning than at the height of growing season. Potential soil drivers (*e.g.* moisture, microbial biomass-N and DON) and plant phenology together drove the seasonal dynamics of inorganic N and amino acid-N. Arginine, histidine, serine, leucine, aspartic acid, glycine, glutamic acid and proline composed the dominant soil amino acid pool in the temperate forest soils. The basic amino acids (arginine and histidine) were consistently dominant irrespective of vegetation and season, suggesting that selective sorption by the soil solid phase could play an important role in regulating the cycling of amino acid-N in these temperate forest ecosystems. This research indicates that

changes in local soil properties, and plant phenology caused by seasonality, exert a powerful influence on the characteristics of plant-soil N cycling.

Keywords: Dominant amino acids; growing season; plant-soil N cycling; soil soluble N; temperate forests

1 Introduction

Soil nitrogen (N) availability often limits the net primary productivity of most terrestrial ecosystems (LeBauer and Treseder 2008). The availability of soil N is increasingly influenced by N deposition (Bobbink et al. 2010; Liu et al. 2013), and understanding the interaction of plant community and soil N cycling is very important. Soil soluble N in either an organic form (*e.g.* amino acids, oligopeptides; Näsholm et al. 1998, 2009), or inorganic form (*e.g.* NH_4^+-N and NO_3^--N) supplies N to plants and their associated symbionts and to the wider soil microbial community. Although plants and microbes can access many forms of N when grown in isolation, in natural systems they optimize their transport systems to reflect the availability of different N sources. This can be viewed as an opportunistic response rather than a preference for different N forms *per se* (Moreau et al. 2019). Further, the pool size and composition of soil soluble N has been reported to regulate the relationship of inter-species competition (Chapin et al. 1993; Harrison et al. 2007; Hill et al. 2011). Hence, the investigation of the seasonal dynamics of soil soluble N across plant communities and the potential drivers, is likely to prove important to understanding ecosystem function. Although related research has been conducted in arctic (Weintraub and Schimel 2005), alpine (Lipson

et al. 1999b) and temperate grassland ecosystems (Warren and Taranto 2010), many gaps in the knowledge on the temporal variability in soil N resources and the potential drivers still remain, especially with respect to temperate forests. In the last two decades, soil amino acid-N has been increasingly recognized as an important source of soluble N to plants (Chapin et al. 1993; Hill et al. 2019b; Näsholm et al. 1998, 2009). The 20 common amino acids that compose protein have different intrinsic properties, *e.g.* molecular weights, structures and side-chain chemistry, which influence the behaviour of amino acid-N in soils (Gonod et al. 2006; Rothstein 2010). Individual amino acids can therefore appear to be dominant in the soil amino acid pool (Björk et al. 2007; Werdin-Pfisterer et al. 2012). Here and throughout the paper the "dominant" soil amino acid means that it can be always detected over time in the ecosystem.

The biochemical properties of soils are strongly influenced by plant communities (Björk et al. 2007; Wang et al. 2006; Werdin-Pfisterer et al. 2009), and it is logical to predict distinct N cycling traits between plant communities. For example, some researchers have reported that the concentration of total soil amino acids in the plant communities of late successional stage forests are higher than that of early successional stages (Kielland et al. 2007; Werdin-Pfisterer et al. 2009). Generally, plant communities can influence the availability of soil soluble N, primarily through the following mechanisms: 1) soil temperature and moisture could be changed by plant cover (D'Odorico et al. 2007; Roberts et al. 2009), potentially influencing the activities of soil enzymes and microorganisms and the production of available

N. In particular, for soil moisture, a higher soil moisture content can increase the accessibility of dissolved organic N (DON), which will be beneficial to the subsequent depolymerization and mineralization of N; 2) the quantity and quality of litter input into soils could differ among plant species. Conifer species are especially notable as they often create strongly acidic soils, which do not favour nitrification (Killham 1990). Further, different foliage litters contain different amounts and kinds of amino acids (Rajendran and Kathiresan 2000), potentially leading to the production or leaching of some individual soil amino acids (Hicks et al. 1991), while others may contain high levels of polyphenolics that repress mineralization (Kraus et al. 2003); 3) the magnitude of N uptake could be species-specific (Kahmen et al. 2006; McKane et al. 2002). Different plant species or their symbionts may prefer to take up specific N forms to satisfy their physiological needs (McKane et al. 2002); 4) roots can excrete organic substances (Kuzyakov et al. 2007), including sugars (50–70% of total exudate), carboxylic acids (20–30%) and amino acids (10–20%), which can stimulate rhizosphere microbial activity and lead to soil organic matter decomposition, releasing organic or inorganic N (Meier et al. 2017; Moreau et al. 2019).

As soil moisture, temperature and plant metabolism are determined by climate, however, the four mechanisms described above are expected to be strongly regulated by season. Over the growing season: first, air temperature and precipitation can change considerably. This is especially true for the temperate forest ecosystems where soil temperature and moisture can be significantly altered (Wang et al. 2006, 2013); second, the input of fresh litter to soils by

plants can change during the year, and N immobilization by soil microbes can be promoted by the input of C-rich plant detritus. The soil microbial biomass can therefore also be expected to fluctuate seasonally (Bardgett et al. 2007; Edwards et al. 2006); third, the amounts and forms of available soil N and its uptake by plants can differ (McKane et al. 2002; Stahl et al. 2011). The seasonal variation in the uptake of soil N by plants is likely to be very strong, being high during the growing season and low when plants are not active (McKane et al. 2002; Weintraub and Schimel 2005); last, the exudation rate of the organic substances by plant roots can also be highly seasonally dynamic (Phillips et al. 2008). Therefore, the production and consumption of soil inorganic N and amino acids are not only influenced by plant community composition, but may also more deeply regulated by season. Under the variation of seasonal climate between years, the temporal dynamics of these soil N sources may even differ within a plant community (Warren and Taranto 2010).

The temperate forest ecosystems in northeast China are very functionally and spatially important, accounting for 35% of the total forest area and 40% of the total forest biomass of China (Wang et al. 2008). Over recent decades, anthropogenically-derived N deposition has been shown to be substantial and has progressively risen in this region (15−50 kg N ha^{-1} yr^{-1}; Liu et al. 2013; Yu et al. 2019). This enhanced N deposition has changed the availability of soil NH_4^+-N and NO_3^--N (Liu et al. 2013), which in turn may strongly influence species composition or community succession (Bobbink et al. 2010; Hill et al. 2011; Stevens et al. 2010). In the context of this, exploring the cycling and feedback of plant-soil N in these

123 forests is important. On a monthly time scale during the growing season (from May to
124 September), the concentrations of soil free amino acid-N, NH_4^+-N, NO_3^--N, DON, microbial
125 biomass-N and a set of soil properties were quantified in typical forest types of northeast
126 China. Using this comprehensive data set, in these forest soils that are rich in organic matter
127 we aimed to explore: (1) the composition of the soil amino acid pool; (2) the dynamics of soil
128 inorganic N and amino acid-N across plant communities and seasons, and their potential
129 drivers. Specifically, we hypothesized that vegetation plays an important role in mediating
130 the pool size and the composition of soil inorganic N and amino acid-N, but that season could
131 be more influential than vegetation in influencing the availability of these soil available N
132 sources in the temperate forest ecosystems.

133 **2 Materials and methods**

134 **2.1 Study sites**

135 Two study sites, Liangshui National Natural Reserve (LNNR; 47°10′N, 128°53′E) and
136 Maoershan Forest Ecosystem Research Station (MFER; 45°24′N, 127°40′E), were used in the
137 study. LNNR is located in the Lesser Khingan Mountains, characterized by a temperate
138 monsoon climate with a mean annual temperature of -0.3 °C. The annual precipitation is 676
139 mm which mainly occurs in summer (Fig. 1). As one of the few virgin temperate forest
140 reserve areas in northeast China, LNNR has a variety of pristine forest types with a known
141 history of community succession and ecozones. Broad-leaved Korean pine (*Pinus koraiensis*)
142 mixed forest represents the typical zonal climax, while the forests of *Picea asperata* and

143 *Abies nephrolepis* usually represent the topographic or edaphic climax at wet or swampy sites.
144 Since the clear-cutting of previous broad-leaved *Pinus koraiensis* mixed forest in the 1950s,
145 secondary birch (*Betula platyphylla*)-dominated stands which represent the naturally
146 regenerating forest type, have been established (see Qi et al. 2014 for more site details).
147 Based on this, four typical forest types were selected here for the study (Table 1).
148 MFER is located about 250 km south-west of LNNR, in the west branch of Zhangguangcai
149 Mountains which belongs to the Changbai Mountain System. Its climate is characterized by a
150 temperate continental monsoon, with a mean annual temperature of 2.8 °C and annual
151 precipitation of 629 mm, of which 80% falls in July and August (Fig. 1). Annual evaporation
152 determined by a Class A evaporation pan is 884 mm (Wang et al. 2006). The zonal climax
153 community here is also broad-leaved *Pinus koraiensis* mixed forest. However, due to strong
154 human interference in or before the 1930s the virgin forests have completely disappeared.
155 The current forest vegetation is dominated by broad-leaved secondary forests, with
156 regenerated *Pinus koraiensis* saplings under the canopy. Large areas of pure broad-leaved
157 and coniferous plantations have also been established. Two typical forest types were chosen
158 for the study at this site (Table 1).

159 **2.2 Soil sampling**

160 A 20 m × 30 m permanent plot was established in each forest type in 2016. Soil samples were
161 collected monthly from early May to late September, and sampling on rainy days or during
162 muddy periods was avoided. The actual sampling dates were, as follows: 6th May, 16th June,

15th July, 19th August and 21st September (for MFER site) or 23rd September (for LNNR site). At each soil sampling time, 30 individual soil cores from the 0–10 cm mineral soil layer were randomly taken within the plot of each forest type. The organic litter layer was removed by hand before coring. The soil cores were cleared of plant debris and stones, placed into a plastic bag, sealed and kept on ice during transport. Two teams simultaneously undertook the field work, sampling on the early morning of each day. The sampling for any forest type was completed within 2 h, followed by immediate transport to the laboratory. When soil samples were brought back to the laboratory, six soil cores were randomly recombined into a composite sample, making five composite samples for each forest type (n=5). These samples were then stored at 4 °C for no more than 48 h.

2.3 Laboratory procedures

Soil samples were sieved to pass 2 mm. Soil $pH_{(water)}$ was measured on a 1:2 (weight/volume) ratio, and moisture content was determined after drying at 105 °C for 24 h. Fresh soil subsamples (4 g) were put into 50 mL centrifuge tubes, followed by the addition of 20 mL of deionized water. The soil–water suspension was shaken at 220 rpm for 1 h, centrifuged at 16000 *g* for 15 min, and then vacuum-filtered through a 0.45 μm filter. The extracts were stored at -20 °C until analysis. The concentrations of individual amino acids in solutions were analyzed by reverse phase high performance liquid chromatography (HPLC) on an LC2000 (Techcomp, China) equipped with a Kromasil C_{18} HPLC column (150 mm × 4.6 mm, 5 μm). 6-aminoquinolyl-N-hyroxy-succinimidyl carbamate was applied to pre-derivatize the soil extract solution (AQC; Liu 1994). The volume of sample injection was 10 μL, and the flow

rate was 1.0 mL/min, with a column temperature of 37 °C and ultraviolet detection at 254 nm.

Soil dissolved N was measured using the potassium persulphate ($K_2S_2O_8$) oxidation method, using distilled water as the extractant. DON was determined by the difference between dissolved N and the resultant dissolved inorganic N (Bardgett et al. 2007). Soil microbial biomass-N was determined by the chloroform fumigation-extraction method (Brookes et al. 1985). Soil NH_4^+ and NO_3^- extracted by 1 mol/L KCl were measured using an AA3 flow injection auto-analyzer (Seal Analytical GmbH, Germany). The soil organic C and total N contents were analyzed using a vario MACRO cube elemental analyzer (Elementar, Germany).

2.4 Statistical analyses

Some amino acids were only detected in a minority of samples across the five months, so only the "dominant" (see *Introduction*) soil amino acids were analyzed statistically as they were deemed to be quantitatively important to the plant-soil system. Repeated measures analysis of variance (ANOVA) was applied to examine the effects of forest type and month on the concentration and the ratio of soil extracted N (SPSS 22.0; SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was performed to identify the variation in soil N characteristics among forest types (OriginPro 2016; Originlab Corp., MA, USA). Multiple regression analysis was used to determine the potential soil factors that drove the variation in the availability of soil soluble N (SPSS 22.0; SPSS Inc., Chicago, IL, USA). The coefficients of variation for soil soluble N, moisture and microbial biomass-N were calculated to compare

the degree of variation between forest type and season, and their differences were tested through independent sample t-test (SPSS 22.0; SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Dynamics of soil soluble N and microbial biomass-N across forest types and months

The concentrations of soil free amino acid-N, NH_4^+-N, NO_3^--N, DON, and microbial biomass-N, showed marked variation among forest types and months (Fig. 2; Table S1). There was a clear interaction between forest type and month (Table S1), with the concentrations of soil soluble N or microbial biomass-N being highly variable from May to September (Fig. 2). However, whether for soil soluble N or microbial biomass-N, the concentrations of N tended to be the highest in May in comparison to the other months, with only a few exceptions (Fig. 2). For secondary *Pinus koraiensis–Betula platyphylla* mixed forest and *Fraxinus mandshurica* plantation, June exhibited the highest concentration of NH_4^+-N while September exhibited the highest concentration of DON (Fig. 2b and d). For *Picea asperata* plantation, July exhibited the highest microbial biomass-N (Fig. 2e). Although the month that showed the lowest concentration of N varied between soil N sources, these were concentrated between June and September (Fig. 2). Free amino acid-N and NO_3^--N appeared to be the lowest in July than in other months, NH_4^+-N appeared to be the lowest mostly in August, DON appeared to the lowest often in September but occasionally in July, and microbial biomass-N showed the lowest concentrations in all months except May (Fig. 2).

3.2 Composition of dominant soil amino acids in temperate forests

Forest type and month also appeared to influence the composition and concentration of the dominant soil amino acids (Fig. 3; Table S1). Generally, eight dominant free amino acids were detected in the temperate forest soils, and their concentrations followed the order: arginine > histidine > serine > leucine > aspartic acid > glycine > glutamic acid > proline (Fig. 3). Except for arginine, histidine and aspartic acid, the other amino acids did not always appear to be dominant across these forest soils (Fig. 3). This was especially obvious for proline as it only appeared to be dominant in *Fraxinus mandshurica* plantation soils over the growing season, with the concentration ranging from 0.10 to 0.26 μg N/g dry soil (Fig. 3h). The concentrations of dominant soil amino acids also tended to be the highest in May than in other months (Fig. 3). Nevertheless, for pristine *Abies nephrolepis* forest and *Picea asperata* plantation, the concentrations of serine, aspartic acid and glutamic acid were the highest in August (Fig. 3c, e and g). For secondary *Pinus koraiensis–Betula platyphylla* mixed forest, the concentrations of glycine and glutamic acid were the highest in September (Fig. 3f and g). The concentrations of dominant soil amino acids were the lowest mostly in July but occasionally in June and August (Fig. 3). Pristine *Abies nephrolepis* forest was the only forest type that showed the lowest concentrations of many dominant soil amino acids (arginine, leucine, aspartic acid and glutamic acid) in June than in other months (Fig. 3a, d, e and g). Meanwhile, in most cases the lowest concentrations were observed in August (Histidine, aspartic acid, glutamic acid and proline) particularly in the secondary *Pinus koraiensis–Betula platyphylla* mixed forest and *Fraxinus mandshurica* plantation (Fig. 3b, e, g and h).

3.3 Potential drivers of variation in soil inorganic N and free amino acids

Based on the stepwise analyses for the data of six forest types over five months, moisture, microbial biomass-N and DON were the most related soil factors that could explain the variation in the abundance of soil total free amino acids, NH_4^+, NO_3^-, arginine, histidine and aspartic acid (Table 2; Fig. 4). Other soil dominant amino acids were not analyzed as they did not occur in appreciable levels in all forest types. Soil total free amino acids, arginine and histidine were positively related to moisture which could explain 48.4%, 29.9% and 50.1% of the variation, respectively (Fig. 4a, d and e). Soil NH_4^+ was positively related to DON which could explain 25.6% of its variation (Fig. 4b). Soil NO_3^- and aspartic acid were both positively related to moisture and microbial biomass-N, which together could explain 31.6% and 46.0% of the variation, respectively (Fig. 4c and f).

3.4 Relationship among amino acid-N, inorganic N, DON and microbial biomass-N

Multivariate correlations among soil amino acid-N, inorganic N, DON and microbial biomass-N were analyzed by PCA (Fig. 5). The two main components together explained 70%, 79%, 77%, 78% and 77% of the variation in sample data for May, June, July, August and September, respectively (Fig. 5). The pattern of correlations of these soil biogenic N forms among the six forest types appeared to vary prominently over time. First, except for a few cases, different forest types showed a scattered pattern within a specific month (Fig. 5). For example, pristine broad-leaved *Pinus koraiensis* mixed forest and *Picea asperata* plantation clustered in September (Fig. 5e). These two forest types showed higher concentrations of soil histidine-N, serine-N, and lower NH_4^+-N, proline-N (not dominant),

268 microbial biomass-N, and DON, which is opposite to *Fraxinus mandshurica* plantation (Fig.
269 5e); secondary *Pinus koraiensis–Betula platyphylla* mixed forest showed lower
270 concentrations of soil free amino acid-N, histidine-N and serine-N (not dominant) (Fig. 5e);
271 pristine *Abies nephrolepis* forest showed higher concentrations of soil glutamic acid-N,
272 glycine-N and leucine-N, whereas secondary *Picea asperata–Betula platyphylla* mixed forest
273 showed higher concentrations of soil NO_3^--N, arginine-N and aspartic acid-N (Fig. 5e).
274 Second, the distribution pattern of these forest types in the PCA appeared to differ over five
275 months. This could be well illustrated as the forest types that possessed similar soil N
276 characteristics (*i.e.* the clustered forest types) changed over time (Fig. 5).

277 **4 Discussion**

278 **4.1 Composition of dominant soil amino acids in temperate forest ecosystems**

279 An increasing body of research has employed amino acids as an organic N source to explore
280 ecologically crucial issues, *e.g.* organic N utilization (Näsholm et al. 1998), N niche
281 differentiation (McKane et al. 2002) and species coexistence (Ouyang et al. 2016). Here we
282 show that arginine, histidine, serine, leucine, aspartic acid, glycine, glutamic acid and proline
283 composed the dominant soil amino acids in the temperate forest ecosystems of northeast
284 China (Fig. 3). This provides a basis for further research that examines the importance of
285 dominant soil amino acid-N in plant nutrition within these forest ecosystems (Gao et al.
286 2019). The availability of soil amino acids is determined by the relative rates of production
287 and consumption; when the production rate exceeds the consumption rate, its availability
288 appears to increase and *vice versa*. The processes related to production and consumption

289 involve complex biochemical mechanisms. First, soil amino acids have various sources. The
290 breakdown of protein probably dominates (Jan et al. 2009), but dry and wet deposition
291 (Mopper and Zika 1987), vegetation stemflow and throughfall (Michalzik and Matzner
292 1999), root exudation and death (Jones and Darrah 1994), microbial turnover, and faunal
293 inputs (Hill et al. 2019a), also contribute. Second, amino acids have many sinks, including
294 the selective use by microorganisms and plants (Endres and Mercier 2003; Lipson et al.
295 1999a), mineralization (Jones and Kielland 2002) and leaching (Fischer et al. 2007). Probably
296 as a result of selective inputs or consumption, soil dominant amino acids appeared to be
297 distinct among forest types (Fig. 3) and also seemed different to the reports from other plant
298 ecosystems (Björk et al. 2007; Werdin-Pfisterer et al. 2012). Different forest types showed
299 different N cycling characteristics (Fig. 5), and the variation in the composition of soil amino
300 acids could partially reflect the variation in soil N cycling traits. This was especially
301 prominent for *Fraxinus mandshurica* plantation, as proline was dominant only in its soils (Fig.
302 3h). Although we cannot explain the phenomenon, it could illustrate that the composition of
303 soil dominant amino acids can indirectly reflect differences in soil-plant N cycling processes
304 between plant communities. Despite these differences in factors related to N cycling and the
305 variation in composition of the pool of amino acids, the basic amino acids, arginine and
306 histidine, appeared to continuously dominate in soils and on average accounted for 26% of
307 the free amino acids. This suggests that selective sorption of amino acids by the soil solid
308 phase could also play an important role in amino acid-N cycling in the temperate forest soils
309 (Rothstein 2010). The soils in the studied region mostly belong to dark brown forest soils,

which are rich in organic matter (Table 1) and can have a high clay content (Zhang and Song 2005). This may partially contribute to the adsorption and prevalence of basic amino acids. Additionally, aspartic acid belongs to the acidic amino acids which generally have a low sorption potential on the soil solid phase relative to basic or neutral amino acids (Gonod et al. 2006), but it also dominated in the soil amino acid pool here (Fig. 3e). Some researchers suggest that severed fine roots and mycorrhizal hyphae (Hobbie and Hobbie 2013) may lead to the prevalence of aspartic acid (Senwo and Tabatabai 1998; Werdin-Pfisterer et al. 2009). As described above, there were many potential factors that could affect the composition of soil dominant amino acids. Based on the data collected here, we cannot explain explicitly which factors induce the difference or similarity in the components of dominant soil amino acids between forest types, but this deserves further research. Additionally, soil soluble N can be primarily divided into three parts, which are mobile in soil solution, adsorbed to ion exchange sites and spatially inaccessible due to isolation in microsites (Darrouzet-Nardi and Weintraub 2014). In contrast to inorganic N (KCl extraction), deionized water as the extractant for soil amino acids, which may underestimate the concentrations of some amino acids that are prone to sorption on the soil's solid phase (*e.g.* histidine and arginine) (Rothstein 2010; Werdin-Pfisterer et al. 2009). Nevertheless, free amino acids may be more easily taken up by roots and microbes, representing more available N sources for organisms than the adsorbed types.

4.2 Seasonal dynamics of soil soluble N and potential drivers

330 Plant community type can significantly influence the turnover of soil C and N through the
331 change of soil biochemical conditions, *e.g.* pH (Killham 1990), the activity of enzymes and
332 the structure of the microbial community (Björk et al. 2007; Weand et al. 2010). The six
333 forest types chosen here were comprised of different plant species, and they were
334 characteristic of different growth stages, soil pH, microbial biomass, ratios of C to N and
335 relative moisture (Table 1; Fig. 2e). Some of the plant communities are in the early
336 successional stage (secondary *Pinus koraiensis–Betula platyphylla* mixed forest, secondary
337 *Picea asperata–Betula platyphylla* mixed forest and *Fraxinus mandshurica* plantation), while
338 the others are in a late successional stage (pristine broad-leaved *Pinus koraiensis* mixed forest,
339 *Picea asperata* plantation and pristine *Abies nephrolepis* forest). As hypothesized, the
340 concentration of soil soluble N was affected by plant communities (Figs. 2a, b, c, d and 3;
341 Table S1). This is in agreement with many studies from taiga forest (Kielland et al. 2007),
342 boreal forest (Werdin-Pfisterer et al. 2009) and tundra (Björk et al. 2007) ecosystems. As
343 there was interaction between forest type and month on the availability of soil soluble N
344 (Table S1), however, the distinction in the concentration of soil soluble N between plant
345 communities varied across months (Figs. 2 and 3). For instance, the concentration of total soil
346 amino acids in May was higher in pristine *Abies nephrolepis* forest than in secondary *Pinus*
347 *koraiensis–Betula platyphylla* mixed forest; in August, this was reversed (Fig. 2a). In contrast
348 to the total soil C or N pool in temperate forests, which is primarily comprised of organic
349 matter and can be stable for years to decades (Lützow et al. 2006), inorganic N and amino
350 acid-N can be strongly affected by many potential factors and can be easily consumed in soil

351 solution (*e.g.* by roots, microbes or enzymes). Compared with season, the type of plant
352 community showed less influence on the availability of soil soluble N in the temperate forest
353 ecosystems of northeast China. This is different to the research from boreal and taiga forest
354 ecosystems where the concentrations of soil amino acids have been reported to increase over
355 the succession sequence of forest communities (Kielland et al. 2007; Werdin-Pfisterer et al.
356 2009). This difference suggests that the turnover characteristics of soil inorganic N or amino
357 acid-N could vary between forest ecosystems in different biomes.
358 In temperate forest ecosystems, belowground biological processes are extremely influenced
359 by the seasonal dynamics of soil temperature and moisture (Wang et al. 2006). From May to
360 September, the air temperature and precipitation changed considerably (Fig. 1), and the
361 characteristics of soil N cycling varied over the growing season (Fig. 5). The stronger
362 influence of season on the availability of soil inorganic N and free amino acids than forest
363 type, was primarily reflected in the following two aspects: 1) coefficients of variation of soil
364 inorganic N, free amino acids and the potential drivers (moisture and DON) tended to be
365 greater for month than for forest type, which was especially prominent for NO_3^- (Table 3). A
366 probable explanation could be because, compared with other forms of N, soil NO_3^- was more
367 prone to leaching induced by the seasonal change of precipitation; and 2) the concentrations
368 of soil inorganic N and free amino acids tended to be higher at the beginning than at the
369 height of growing season due to increased plant demand. From the onset to the height of
370 growing season, the concentration of soil free amino acid-N, NH_4^+-N and NO_3^--N decreased
371 by 62%, 57% and 72%, respectively (Fig. 6a, b and c). Both abiotic and biotic factors could

possibly lead to this phenomenon. The precipitation mostly occurred at the height of growing season (Fig. 1), which could cause the leaching of soil soluble N especially for those that are not readily adsorbed on the soil solid phase, *e.g.* acidic amino acids (Gonod et al. 2006) and NO_3^-. However, as rainy days or muddy periods were avoided during soil sampling, so the lower concentration of soil inorganic N, free amino acids and DON could not be directly explained by the influence of precipitation. Based on stepwise analyses, the variations of soil inorganic N and amino acids across forest types and months were potentially driven most by soil moisture, followed by microbial biomass-N and DON (avg. 7.7% of which was free amino acid-N), and the availability of inorganic N and amino acids were positively related to these potential drivers (Table 2; Fig. 4). At the beginning of the growing season, the concentration of soil moisture, microbial biomass-N and DON was 58%, 53% and 113% higher than that at the height of growing season, respectively (Fig. 6d, e and f). In the early May of northeast China, it was shortly after the periods of soil freezing and thawing (Wang et al. 2013) which could release high amounts of water and N nutrients to the soil solution (Grogan et al. 2014; Sanders-DeMott et al. 2018). The moist conditions could strengthen the accessibility of DON sources to soil enzymes and microbes (Darrouzet-Nardi and Weintraub 2014), promoting the depolymerization and mineralization of N in soil solution. Therefore, as reported by others (Bardgett et al. 2007; Hackl et al. 2004), we also suggest that soil moisture is a very important factor for mediating the belowground processes related to soil N sources. On the other hand, both the uptake of N by soil microbes and plant roots could also play an important role in mediating the seasonal dynamics pattern of soil inorganic N and amino

393 acids. Nevertheless, the concentration of soil microbial biomass-N also decreased by 35%
394 from the onset to the height of growing season (Fig. 6f). As a storage pool of soil N, the
395 turnover of microbial N is frequently very rapid (three to five days; Kuzyakov and Xu 2013).
396 The available soil N will ultimately flow into the plants as they are more stable N storage
397 pool (Kuzyakov and Xu 2013). The phenology of plants is strongly influenced by seasonality,
398 driving the dynamic uptake of soil soluble N by roots (McKane et al. 2002; Stahl et al. 2011).
399 During the onset of the growing season, the N needed for plant growth can partially come
400 from the remobilization of plants' stored resources, and tree growth often starts before N
401 uptake by roots, reducing the N demand from the soils (Millard and Grelet 2010). Meanwhile,
402 the production of roots in the typical temperate forests of these regions can increase
403 significantly during the height of growing season (Quan et al. 2010). This can significantly
404 increase the uptake capacity and support the growth demand of plants for the limited N
405 resource. Thus, the reduction in the pools of available soil N during the height of growing
406 season in the temperate forest ecosystems most-likely results from the higher demand for N
407 nutrient by plants (Weintraub and Schimel 2005).

408 **5 Conclusions**

409 In the temperate forest ecosystems of northeast China, the pool size and composition of soil
410 soluble N were influenced more strongly by season than by vegetation. Soil potential drivers
411 (*e.g.* moisture, microbial biomass-N, DON) and the phenology of plants together drove the
412 seasonal dynamics of soil inorganic N and amino acid-N. Arginine, histidine, serine, leucine,
413 aspartic acid, glycine, glutamic acid and proline composed the dominant soil amino acids in

these temperate forest ecosystems; regardless of vegetation and season, however, basic amino acids (arginine and histidine) dominated consistently, illustrating the potentially important role of selective sorption by the soil solid phase in mediating soil amino acid-N cycling.

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418 **References**

419 Bardgett RD, Mommer L, Vries FTD (2014) Going underground: root traits as drivers
420 of ecosystem processes. Trends Ecol Evol 29: 692−699.
421 https://doi.org/10.1016/j.tree.2014.10.006

422 Bardgett RD, Wal RVD, Jónsdóttir IRS, Quirk H, Dutton S (2007) Temporal variability
423 in plant and soil nitrogen pools in a high-Arctic ecosystem. Soil Biol Biochem
424 39: 2129−2137. https://doi.org/10.1016/j.soilbio.2007.03.016

425 Björk RG, Klemedtsson L, Molau U, Harndorf J, Ödman A, Giesler R (2007) Linkages
426 between N turnover and plant community structure in a tundra landscape. Plant
427 Soil 294: 247−261. https://doi.org/ 10.1007/s11104-007-9250-4

428 Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante
429 M, Cinderby S, Davidson E, Dentener F (2010) Global assessment of nitrogen
430 deposition effects on terrestrial plant diversity: a synthesis. Ecol Appl 20:
431 30−59. https://doi.org/10.1890/08-1140.1

432 Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and
433 the release of soil nitrogen: A rapid direct extraction method to measure
434 microbial biomass nitrogen in soil. Soil Biol Biochem 17: 837−842.
435 https://doi.org/10.1016/0038-0717(85)90144-0

436 Chapin III FS, Moilanen L, Kielland K (1993) Preferential use of organic nitrogen for
437 growth by a non-mycorrhizal arctic sedge. Nature 361: 150−153.
438 https://doi.org/10.1038/361150a0

439 Darrouzet-Nardi A, Weintraub MN (2014) Evidence for spatially inaccessible labile N
440 from a comparison of soil core extractions and soil pore water lysimetry. Soil
441 Biol Biochem 73: 22−32. https://doi.org/10.1016/j.soilbio.2014.02.010

D'Odorico P, Caylor K, Okin GS, Scanlon TM (2007) On soil moisture–vegetation feedbacks and their possible effects on the dynamics of dryland ecosystems. J Geophys Res-BioBgeo 112: G04010. https://doi.org/10.1029/2006JG000379

Edwards KA, Mcculloch J, Kershaw GP, Jefferies RL (2006) Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. Soil Biol Biochem 38: 2843−2851. https://doi.org/10.1016/j.soilbio.2006.04.042

Endres L, Mercier H (2003) Amino acid uptake and profile in bromeliads with different habits cultivated in vitro. Plant Physiol Bioch 41: 181−187. https://doi.org/10.1016/S0981-9428(02)00025-6

Fischer H, Meyer A, Fischer K, Kuzyakov Y (2007) Carbohydrate and amino acid composition of dissolved organic matter leached from soil. Soil Biol Biochem 39: 2926−2935. https://doi.org/10.1016/j.soilbio.2007.06.014

Gao L, Cui X, Hill PW, Guo Y (2019) Uptake of various nitrogen forms by co-existing plant species in temperate and cold-temperate forests in northeast China. Appl Soil Ecol 147: 103398. https://doi.org/10.1016/j.apsoil.2019.103398

Gonod LV, Jones DL, Chenu C (2006) Sorption regulates the fate of the amino acids lysine and leucine in soil aggregates. Eur J Soil Sci 57: 320−329. https://doi.org/10.1111/j.1365-2389.2005.00744.x

Grogan P, Michelsen A, Ambus P, Jonasson S (2004) Freeze–thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. Soil Biol Biochem 36: 641−654. https://doi.org/10.1016/j.soilbio.2003.12.007

Hackl E, Bachmann G, Zechmeister-Boltenstern S (2004) Microbial nitrogen turnover in soils under different types of natural forest. Forest Ecol Manag 188: 101−112. https://doi.org/10.1016/j.foreco.2003.07.014

Harrison KA, Bol R, Bardgett RD (2007) Preferences for different nitrogen forms by coexisting plant species and soil microbes. Ecology 88: 989−999. https://doi.org/10.1890/06-1018

Hicks RE, Lee C, Marinucci AC (1991) Loss and recycling of amino acids and protein from smooth cordgrass (*Spartina alterniflora*) litter. Estuar Coast 14: 430−439. https://doi.org/10.2307/1352267

Hill EJ, Jones DL, Paterson E, Hill PW (2019a) Hotspots and hot moments of amino acid N in soil: Real-time insights using continuous microdialysis sampling. Soil Biol Biochem 131: 40−43. https://doi.org/10.1016/j.soilbio.2018.12.026

Hill PW, Broughton R, Bougoure J, Havelange W, Newsham KK, Grant H, Murphy DV, Clode P, Ramayah S, Marsden KA, Quilliam RS, Roberts P, Brown C, Read DJ, Deluca TH, Bardgett RD, Hopkins DW, Jones DL (2019b) Angiosperm symbioses with non-mycorrhizal fungal partners enhance N acquisition from ancient organic matter in a warming maritime Antarctic. Ecol Lett 22: 2111−2119. https://doi.org/10.1111/ele.13399

Hill PW, Farrar J, Roberts P, Farrell M, Grant H, Newsham KK, Hopkins DW, Bardgett RD, Jones DL (2011) Vascular plant success in a warming Antarctic may be due to efficient nitrogen acquisition. Nat Clim Change 8: 613−619. https://doi.org/10.1038/nclimate1060

Hobbie JE, Hobbie EA (2013) Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. Front Microbiol 4: 87−100. https://doi.org/10.1016/j.soilbio.2015.07.021

Jan M, Roberts P, Tonheim S, Jones D (2009) Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biol Biochem 41: 2272−2282. https://doi.org/10.1016/j.soilbio.2009.08.013

Jones D, Darrah P (1994) Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. Plant Soil 163: 1−12. https://doi.org/10.1007/BF00033935

Jones DL, Kielland K (2002) Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. Soil Biol Biochem 34: 209−219. https://doi.org/10.1016/S0038-0717(01)00175-4

Kahmen A, Renker C, Unsicker SB, Buchmann N (2006) Niche complementarity for nitrogen: an explanation for the biodiversity and ecosystem functioning relationship? Ecology 87: 1244−1255. https://doi.org/10.1890/0012-9658(2006)87[1244:NCFNAE]2.0.CO;2

Kielland K, McFarland JW, Ruess RW, Olson K (2007) Rapid cycling of organic nitrogen in taiga forest ecosystems. Ecosystems 10: 360−368. https://doi.org/10.1007/s10021-007-9037-8

Killham K (1990) Nitrification in coniferous forest soils. Plant Soil 128: 31−44. https://doi.org/10.1007/BF00009394

Kraus TEC, Dahlgren RA, Zasoski RJ (2003) Tannins in nutrient dynamics of forest ecosystems - a review. Plant Soil 256: 41−66. https://doi.org/10.1023/A:1026206511084

Kuzyakov Y, Hill PW, Jones DL (2007) Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. Plant Soil 290: 293−305. https://doi.org/10.1007/s11104-006-9162-8

Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol 198: 656−669. https://doi.org/10.1111/nph.12235

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89: 371−379. https://doi.org/10.1890/06-2057.1

Lipson DA, Raab TK, Schmidt SK, Monson RK (1999a) Variation in competitive abilities of plants and microbes for specific amino acids. Biol Fert Soils 29: 257−261. https://doi.org/10.1007/s003740050550

Lipson DA, Schmidt SK, Monson RK (1999b) Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80: 1623−1631. https://doi.org/10.1890/0012-9658(1999)080[1623:LBMPDA]2.0.CO;2

Liu HJ (1994) Determination of amino acids by precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and high-performance liquid chromatography with ultraviolet detection. J Chromatogr A 670: 59−66. https://doi.org/10.1016/0021-9673(94)80280-7

Liu X, Zhang Y, Han W, Tang A, Shen J, Cui Z, Vitousek P, Erisman JW, Goulding K, Christie P (2013) Enhanced nitrogen deposition over China. Nature 494: 459−462. https://doi.org/10.1038/nature11917

Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions–a review. Eur J Soil Sci 57: 426−445.

McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415: 68−71. https://doi.org/10.1038/415068a

Meier IC, Finzi AC, Phillips RP (2017) Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biol Biochem 106: 119−128. https://doi.org/10.1016/j.soilbio.2016.12.004

Michalzik B, Matzner E (1999) Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. Eur J Soil Sci 50: 579−590. https://doi.org/10.1046/j.1365-2389.1999.00267.x

Millard P, Grelet G-a (2010) Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. Tree Physiol 30: 1083-1095. https://doi.org/10.1093/treephys/tpq042

Mopper K, Zika RG (1987) Free amino acids in marine rains: evidence for oxidation and potential role in nitrogen cycling. Nature 325: 246−249. https://doi.org/10.1038/325246a0

Moreau D, Bardgett RD, Finlay RD, Jones DL, Philippot L (2019) A plant perspective on nitrogen cycling in the rhizosphere. Funct Ecol 33: 540−552. https://doi.org/10.1111/1365-2435.13303

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392: 914−916. https://doi.org/10.1038/31921

Näsholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. New Phytol 182: 31−48. https://doi.org/10.1111/j.1469-8137.2008.02751.x

Ouyang S, Tian Y, Liu Q, Zhang L, Wang R, Xu X (2016) Nitrogen competition between three dominant plant species and microbes in a temperate grassland. Plant Soil 408: 121−132. https://doi.org/10.1007/s11104-016-2904-3

563 Phillips RP, Erlitz Y, Bier R, Bernhardt ES (2008) New approach for capturing soluble
564 root exudates in forest soils. Funct Ecol 22: 990−999.
565 https://doi.org/10.1111/j.1365-2435.2008.01495.x
566 Qi Y, Li F, Liu Z, Jin G (2014) Impact of understorey on overstorey leaf area index
567 estimation from optical remote sensing in five forest types in northeastern
568 China. Agr Forest Meteorol 198: 72−80.
569 https://doi.org/10.1016/j.agrformet.2014.08.001
570 Quan X, Wang C, Zhang Q, Wang X, Luo Y, Bond-Lamberty B (2010) Dynamics of
571 fine roots in five Chinese temperate forests. J Plant Res 123: 497−507.
572 https://doi.org/10.1007/s10265-010-0322-9
573 Rajendran N, Kathiresan K (2000) Biochemical changes in decomposing leaves of
574 mangroves. Chem Ecol 17: 91−102.
575 https://doi.org/10.1080/02757540008037664
576 Read D, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems−a
577 journey towards relevance? New Phytol 157: 475−492.
578 https://doi.org/10.1046/j.1469-8137.2003.00704.x
579 Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47: 376−391.
580 https://doi.org/10.1007/BF01972080
581 Roberts P, Newsham KK, Bardgett RD, Farrar JF, Jones DL (2009) Vegetation cover
582 regulates the quantity, quality and temporal dynamics of dissolved organic
583 carbon and nitrogen in Antarctic soils. Polar Biol 32: 999−1008.
584 https://doi.org/10.1007/s00300-009-0599-0
585 Rothstein DE (2010) Effects of amino-acid chemistry and soil properties on the
586 behavior of free amino acids in acidic forest soils. Soil Biol Biochem 42:
587 1743−1750. https://doi.org/10.1016/j.soilbio.2010.06.011

588 Sanders-DeMott R, Sorensen PO, Reinmann AB, Templer PH (2018) Growing season
589 warming and winter freeze–thaw cycles reduce root nitrogen uptake capacity
590 and increase soil solution nitrogen in a northern forest ecosystem.
591 Biogeochemistry 137: 337−349. https://doi.org/10.1007/s10533-018-0422-5
592 Senwo ZN, Tabatabai MA (1998) Amino acid composition of soil organic matter. Biol
593 Fert Soils 26: 235−242. https://doi.org/10.1007/s003740050373
594 Stahl VM, Beyschlag W, Werner C (2011) Dynamic niche sharing in dry acidic
595 grasslands-a ^{15}N-labeling experiment. Plant Soil 344: 389−400.
596 https://doi.org/10.1007/s11104-011-0758-2
597 Stevens CJ, Duprè C, Dorland E, Gaudnik C, Gowing DJG, Bleeker A, Diekmann M,
598 Alard D, Bobbink R, Fowler D (2010) Nitrogen deposition threatens species
599 richness of grasslands across Europe. Environ Pollut 158: 2940−2945.
600 https://doi.org/10.1016/j.envpol.2010.06.006
601 Stone MM, Weiss MS, Goodale CL, Adams MB, Fernandez IJ, German DP, Allison SD
602 (2012) Temperature sensitivity of soil enzyme kinetics under N-fertilization in
603 two temperate forests. Global Change Biol 18: 1173−1184.
604 https://doi.org/10.1111/j.1365-2486.2011.02545.x
605 Wang C, Han Y, Chen J, Wang X, Zhang Q, Bond-Lamberty B (2013) Seasonality of
606 soil CO_2 efflux in a temperate forest: Biophysical effects of snowpack and
607 spring freeze–thaw cycles. Agr Forest Meteorol 177: 83−92.
608 https://doi.org/10.1016/j.agrformet.2013.04.008
609 Wang C, Yang J, Zhang Q (2006) Soil respiration in six temperate forests in China.
610 Global Change Biol 12: 2103−2114.
611 https://doi.org/10.1111/j.1365-2486.2006.01234.x

612 Wang X, Fang J, Zhu B (2008) Forest biomass and root–shoot allocation in northeast
613 China. Forest Ecol Manag 255: 4007−4020.
614 https://doi.org/10.1016/j.foreco.2008.03.055
615 Warren CR, Taranto MT (2010) Temporal variation in pools of amino acids, inorganic
616 and microbial N in a temperate grassland soil. Soil Biol Biochem 42: 353−359.
617 https://doi.org/10.1016/j.soilbio.2009.11.017
618 Weand MP, Arthur MA, Lovett GM, McCulley RL, Weathers KC (2010) Effects of tree
619 species and N additions on forest floor microbial communities and extracellular
620 enzyme activities. Soil Biol Biochem 42: 2161−2173.
621 https://doi.org/10.1016/j.soilbio.2010.08.012
622 Weintraub MN, Schimel JP (2005) The seasonal dynamics of amino acids and other
623 nutrients in Alaskan Arctic tundra soils. Biogeochemistry 73: 359−380.
624 https://doi.org/10.1007/s10533-004-0363-z
625 Werdin-Pfisterer NR, Kielland K, Boone RD (2009) Soil amino acid composition across
626 a boreal forest successional sequence. Soil Biol Biochem 41: 1210−1220.
627 https://doi.org/10.1016/j.soilbio.2009.03.001
628 Werdin-Pfisterer NR, Kielland K, Boone RD (2012) Buried organic horizons represent
629 amino acid reservoirs in boreal forest soils. Soil Biol Biochem 55: 122−131.
630 https://doi.org/10.1016/j.soilbio.2012.06.012
631 Yu G, Jia Y, He N, Zhu J, Chen Z, Wang Q, Piao S, Liu X, He H, Guo X, Wen Z, Li P,
632 Ding G, Goulding K (2019) Stabilization of atmospheric nitrogen deposition in
633 China over the past decade. Nat Geosci 12: 424−429.
634 https://doi.org/10.1038/s41561-019-0352-4

635 Zhang L, Song F (2005) Sorption and desorption characteristics of cadmium by four
636 different soils in northeast China. Chinese Geogr Sci 15: 343−347.
637 http://egeoscien.neigae.ac.cn/EN/Y2005/V15/I4/343
638
639

Table 1 Selected characteristics of the study sites

	Forest			Topography				Soil							
Location	Type	Stand age (y)	Canopy closure	Elevation (m)	Slope position	Aspect	Slope (°)	Soil type (CST)	O hr. thk. (cm)	A hr. thk. (cm)	Organic C (g/kg)	Total N (g/kg)	C/N	pH	Relative moisture (%)
LNNR	PP	>250	0.80	436	Mid	SW	17	Bori-Udic Cambosols	5.0	18	96.2	5.97	16.1	5.79	58
	AN	>120	0.90	302	Valley	-	0	Molli-Orthic Gleyosols	6.5	12	71.4	4.41	17.6	5.66	90
	PB	55	0.75	374	Down	SW	10	Bori-Udic Cambosols	4.0	22	96.2	7.55	13.2	5.89	66
	PA	62	0.95	337	Down	W	3	Bori-Udic Cambosols	5.5	20	91.0	5.44	17.1	5.75	69
MFER	SP	55	0.95	385	Mid	SE	15	Bori-Udic Cambosols	3.5	15	86.2	5.32	17.4	6.10	52
	FP	50	0.85	332	Down	W	5	Bori-Udic Cambosols	2.5	20	105.1	9.81	11.0	6.19	56

Notes: Liangshui National Natural Reserve (LNNR); Maoershan Forest Ecosystem Research Station (MFER); pristine broad-leaved *Pinus koraiensis* mixed forest (PP), composed of 70% pine + 30% broad-leaved; pristine *Abies nephrolepis* forest (AN), composed of 90% fir + 10% birch; secondary *Picea asperata–Betula platyphylla* mixed forest (PB), composed of 85% birch + 15% spruce; *Picea asperata* plantation (PA), with less than 10% birch invaded; secondary *Pinus koraiensis–Betula platyphylla* mixed forest (SP), the broad-leaved secondary forest with regenerated *Pinus koraiensis* saplings under canopy; *Fraxinus mandshurica* plantation (FP); southwest (SW); west (W); Chinese Soil Taxonomy (CST); O horizon thickness (O hr.thk.); A horizon thickness (A hr.thk.). Relative moisture represents the ratio of field soil moisture content (w %) to saturated moisture content (w %). Soil organic C, total N, C/N, pH, and relative humidity were determined with 0-10 cm soil, and are means across 5 months (n=25).

Table 2 Results of stepwise regression analysis.

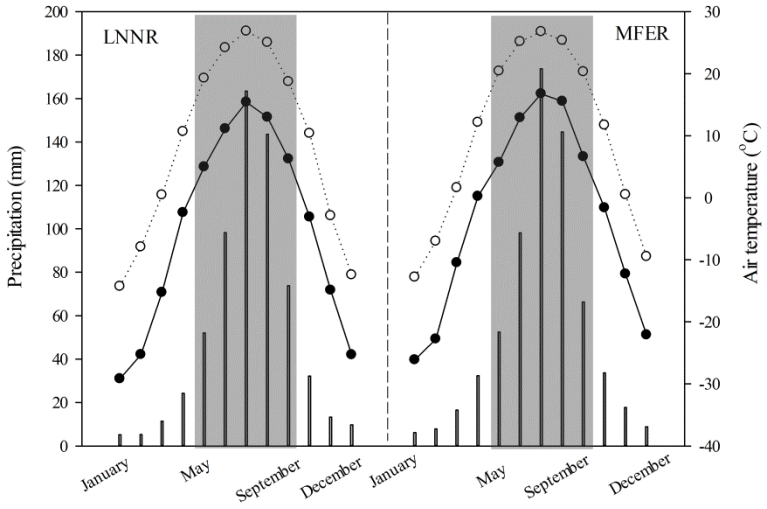
Dependent variable	Variables entered	Sum of squares	df	*F*	*P*
FAA	Moisture content	202.0	1	28.3	< 0.001
NH_4^+	DON	297.1	1	10.9	0.003
NO_3^-	N_{mic} and moisture content	194.2	2	7.7	0.002
Arginine	Moisture content	2.9	1	13.7	0.001
Histidine	Moisture content	3.0	1	31.2	< 0.001
Aspartic acid	Moisture content and N_{mic}	1.4	2	13.7	< 0.001

Notes: Total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N (N_{mic}). Independent variables analyzed for stepwise regression analysis includes: organic C, N_{mic}, pH, total N, DON, moisture content and C/N ratio.

654 **Table 3** Coefficients of variation of soil soluble N, moisture
655 and microbial biomass-N across forest types and months.

	Coefficients of variation (%)				
Soluble N	Forest type	Month	*df*	*t*	*P*
FAA	50 (26)	67 (22)	9	1.17	0.273
NH_4^+	55 (14)	72 (31)	9	1.17	0.271
NO_3^-	50 (8)	77 (20)	9	2.85	0.019
DON	39 (31)	61 (13)	9	1.59	0.145
Arginine	54 (24)	77 (30)	9	1.39	0.199
Histidine	58 (31)	72 (20)	9	0.87	0.407
Serine	62 (42)	94 (20)	7	1.37	0.214
Leucine	60 (23)	83 (12)	8	1.99	0.082
Aspartic acid	69 (35)	82 (30)	9	0.70	0.502
Glycine	52 (38)	83 (35)	6	1.16	0.292
Glutamic acid	57 (30)	79 (40)	7	0.95	0.375
Moisture	21 (9)	29 (8)	9	1.64	0.136
N_{mic}	44 (14)	38 (15)	9	− 0.73	0.483

656 Notes: Total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N
657 (N_{mic}). Means (standard deviations) are shown.
658



659 **Fig.1** Monthly averages of precipitation (bars), minimum (filled circles) and maximum (open circles)
660 air temperature in 2016 for Liangshui National Natural Reserve (LNNR) and Maoershan Forest
661 Ecosystem Research Station (MFER). The shaded area denotes the period of soil sampling. Data was
662 obtained from the local weather station for each site.

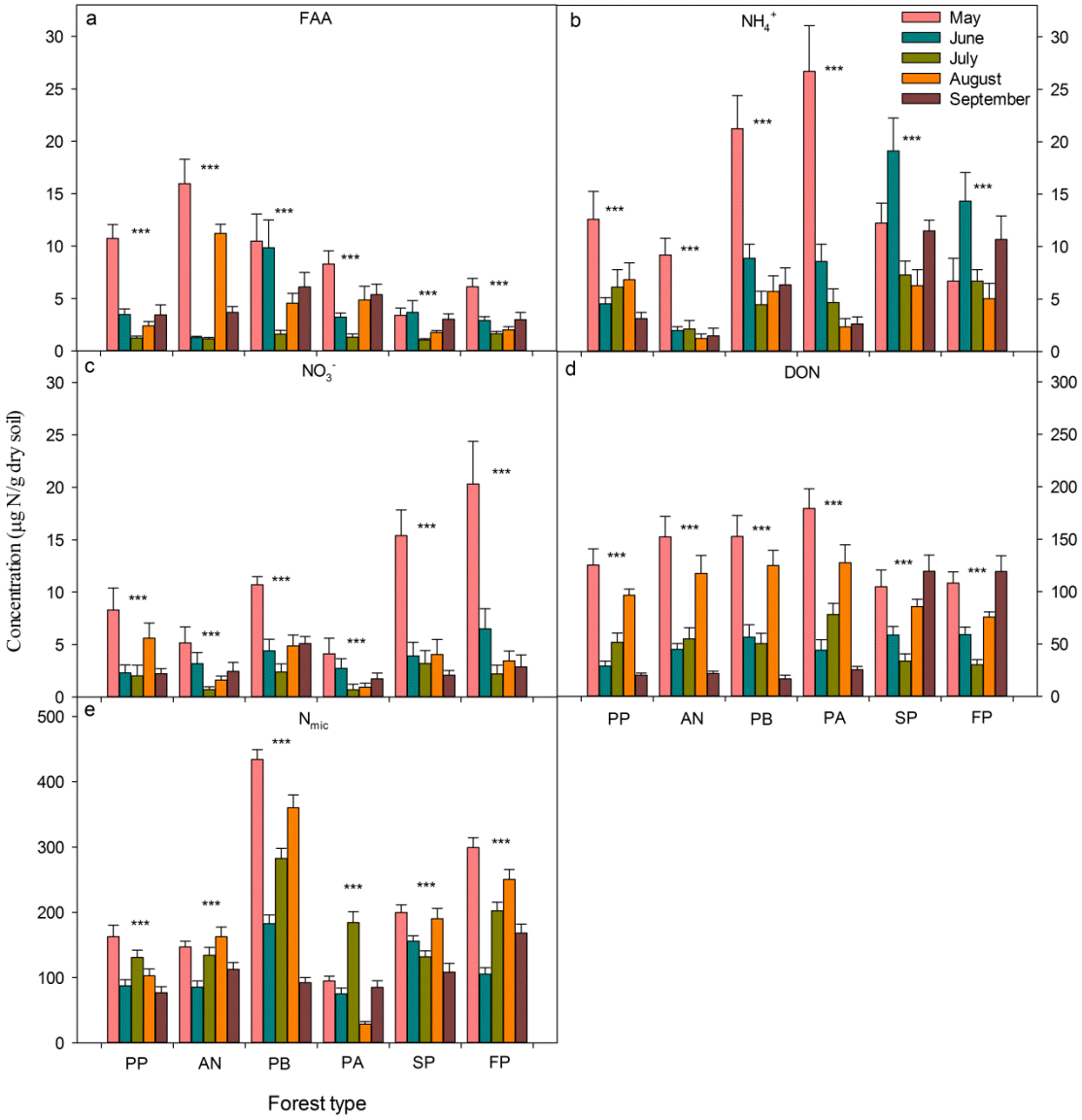


Fig. 2 Concentrations of soil total free amino acid-N (FAA; a), NH_4^+-N (b), NO_3^--N (c), dissolved organic N (DON; d) and microbial biomass-N (N_{mic}; e) over five months. Error bars show standard deviations (n=5). Pristine broad-leaved *Pinus koraiensis* mixed forest (PP); pristine *Abies nephrolepis* forest (AN); secondary *Picea asperata–Betula platyphylla* mixed forest (PB); *Picea asperata* plantation (PA); secondary *Pinus koraiensis–Betula platyphylla* mixed forest (SP); *Fraxinus mandshurica* plantation (FP). *** indicates significant difference between months within a forest at the $p < 0.001$ level.

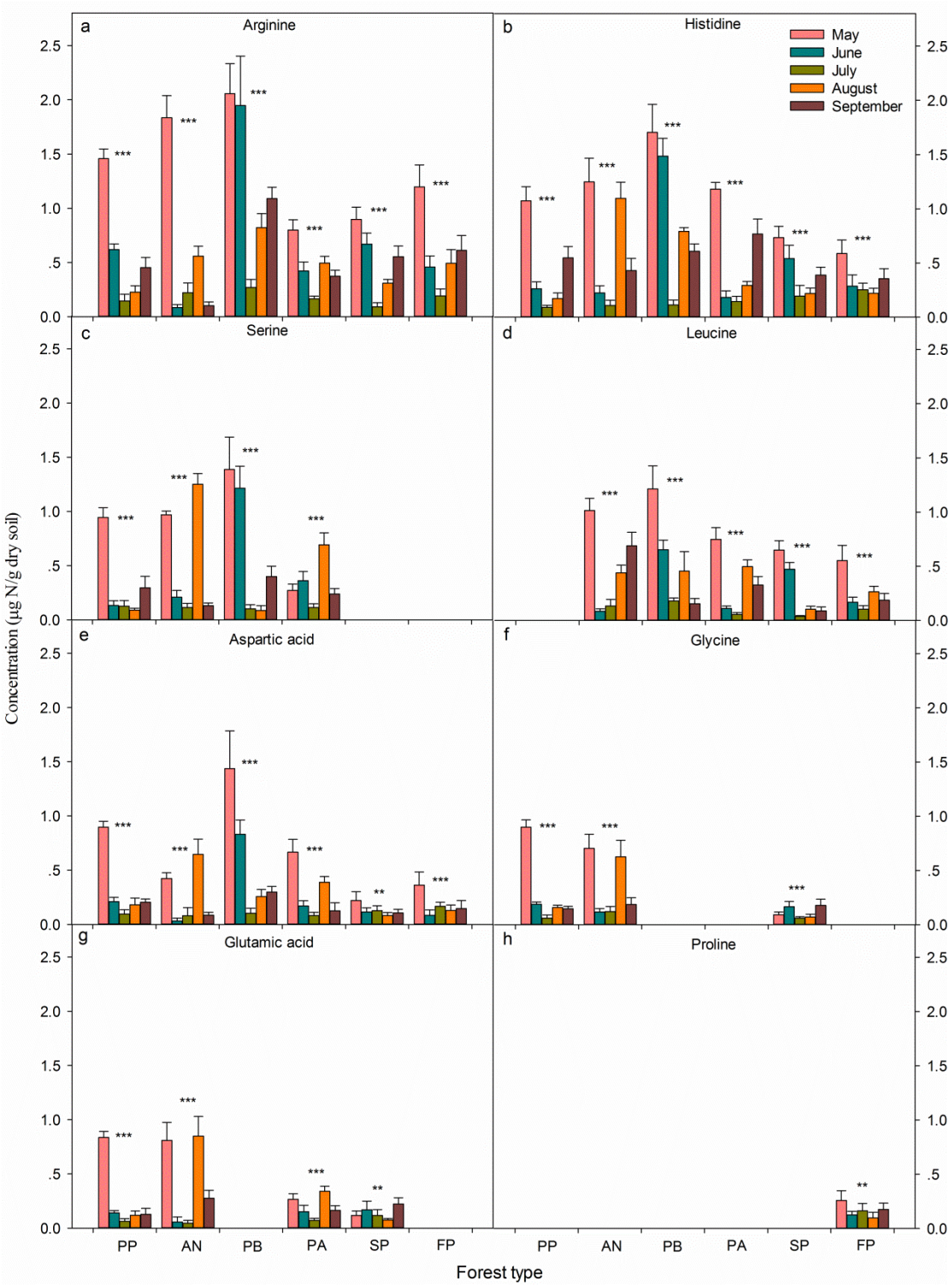
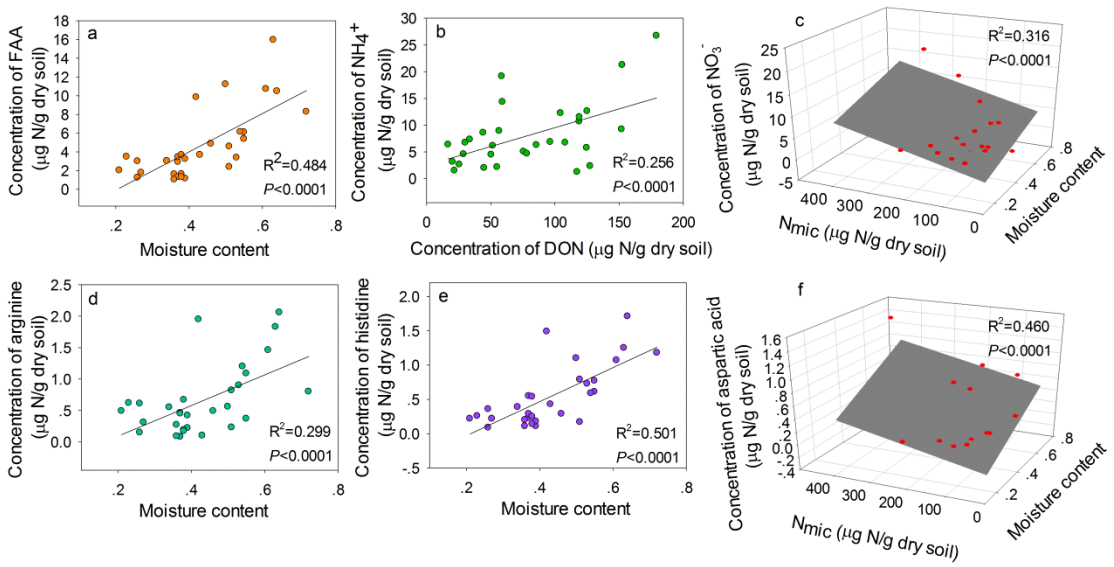


Fig. 3 Concentrations of dominant soil free amino acids in pristine broad-leaved *Pinus koraiensis* mixed forest (PP), pristine *Abies nephrolepis* forest (AN), secondary *Picea asperata–Betula platyphylla* mixed forest (PB), *Picea asperata* plantation (PA), secondary *Pinus koraiensis–Betula platyphylla* mixed forest (SP), and *Fraxinus mandshurica* plantation (FP) over the growing season. May (5); June (6); July (7); August (8); September (9). Error bars show standard deviations (n=5). ** and *** indicates significant difference between months within a forest at the $p < 0.01$ and 0.001

level, respectively.



677 **Fig. 4** Relationships between soil soluble N (total free amino acids, FAA, a; NH_4^+, b; NO_3^-, c;
678 arginine, d; histidine, e; aspartic acid, f) and the related factors obtained from stepwise regression
679 analyses. Microbial biomass-N, N_{mic}. Each colored circle indicates the monthly mean of a forest type
680 (*n*=30).

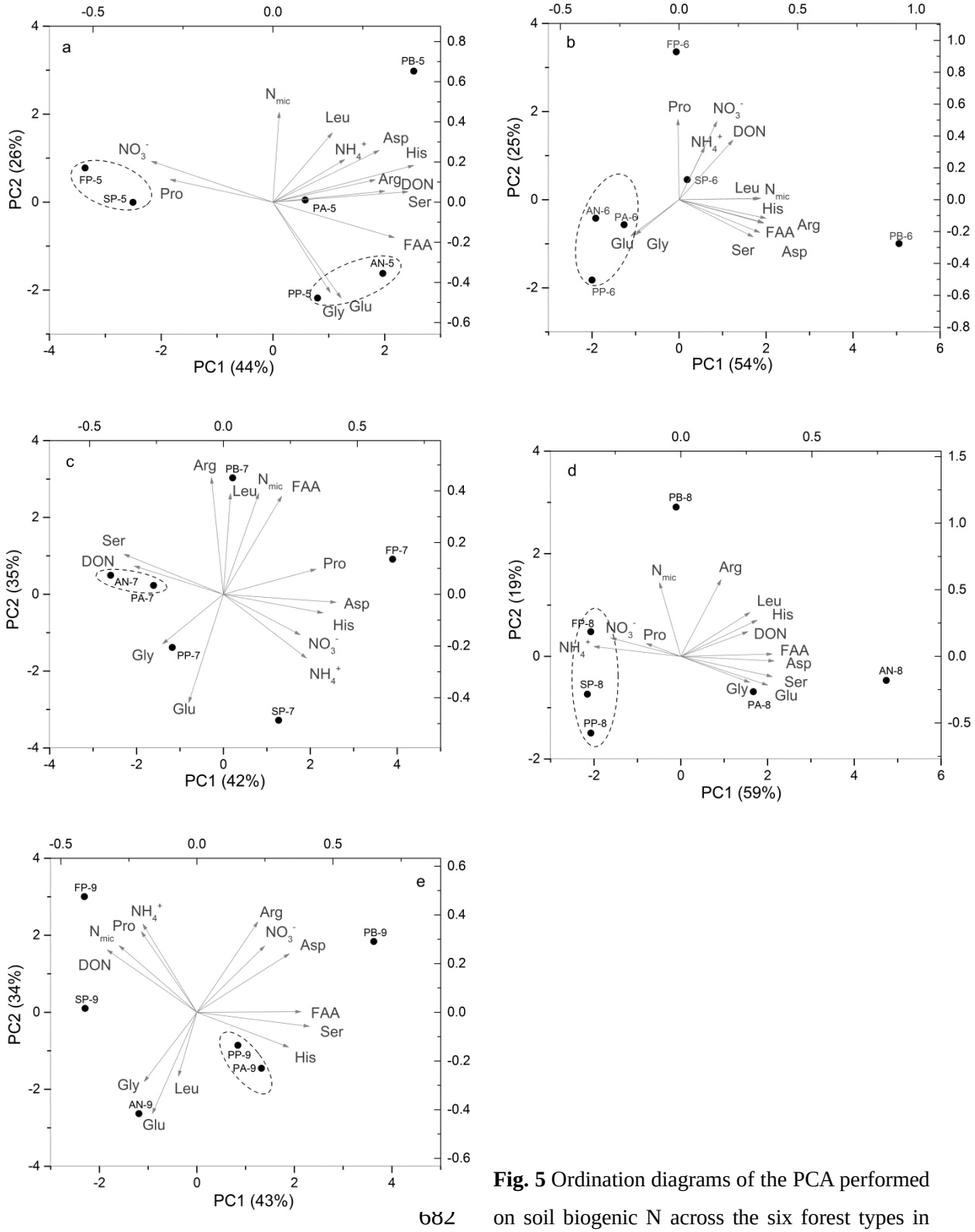
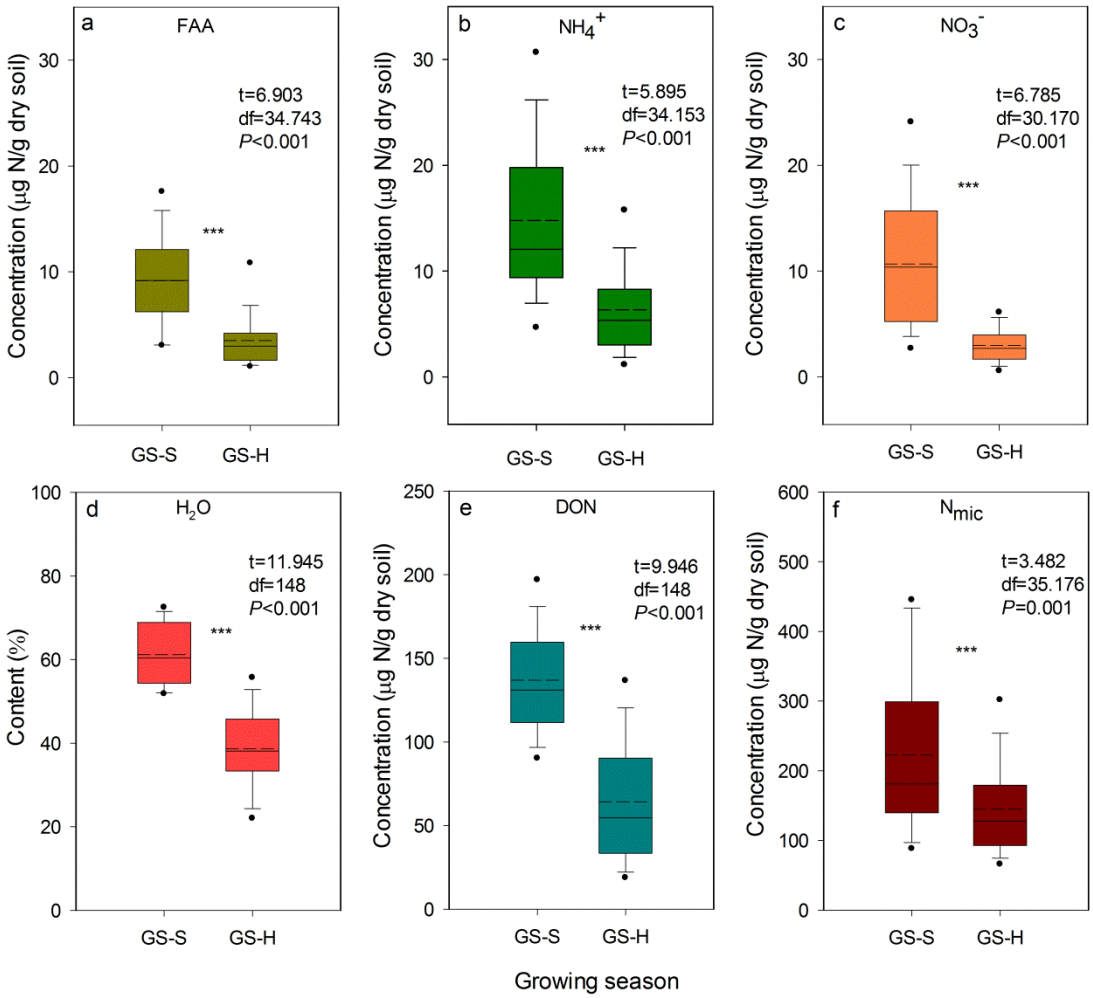


Fig. 5 Ordination diagrams of the PCA performed on soil biogenic N across the six forest types in May (a), June (b), July (c), August (d), and September (e). Pristine broad-leaved *Pinus koraiensis* mixed forest (PP); pristine *Abies nephrolepis* forest (AN); secondary *Picea asperata–Betula platyphylla* mixed forest (PB); *Picea asperata* plantation (PA); secondary *Pinus koraiensis–Betula platyphylla* mixed forest (SP); *Fraxinus mandshurica* plantation (FP). Arginine (Arg); Histidine

(His); Serine (Ser); Leucine (Leu); Aspartic acid (Asp); Glycine (Gly); Glutamic acid (Glu); Proline (Pro); total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N (N_{mic}). Numbers linked with forest types indicate corresponding months. Forest types showing similar soil N characteristics are indicated by the ovals.



691 **Fig. 6** Concentrations of soil total free amino acid-N (FAA; a), NH_4^+-N (b), NO_3^--N (c), moisture
692 (H_2O; d), dissolved organic N (DON; e) and microbial biomass-N (N_{mic}; f) in temperate forests
693 during the start of growing season (GS-S; May; *n*=30) and the height of growing season (GS-H; June,
694 July, August and September; *n*=120). The boundaries of the box indicate the 25% and 75%
695 percentiles, the whiskers indicate the 10% and 90% percentiles, and the points indicate the 5% and
696 95% percentiles, respectively. The solid line in the box marks the median, and the dotted line marks
697 the mean. *** indicates significant difference at the $p < 0.001$ level.
698

699 **Table S1** Repeated measures analysis of variance for the effects of forest type
700 and month on the concentration of soil extracted N.

N source	Forest type			Month			Forest type × Month		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
FAA	5	46.1	< 0.001	1.8	230.0	< 0.001	8.8	33.1	< 0.001
NH_4^+	5	16.6	< 0.001	2.3	444.0	< 0.001	11.4	88.5	< 0.001
NO_3^-	5	15.6	< 0.001	1.8	475.7	< 0.001	8.8	44.9	< 0.001
DON	5	3.8	< 0.011	2.3	944.5	< 0.001	11.4	97.7	< 0.001
Arginine	5	147.3	< 0.001	2.4	303.5	< 0.001	11.9	25.9	< 0.001
Histidine	5	111.8	< 0.001	2.4	298.1	< 0.001	12.0	37.9	< 0.001
Serine	3	51.8	< 0.001	2.5	170.0	< 0.001	7.6	80.9	< 0.001
Leucine	4	39.6	< 0.001	2.7	261.2	< 0.001	10.6	26.1	< 0.001
Aspartic acid	5	144.9	< 0.001	1.8	156.5	< 0.001	8.9	28.3	< 0.001
Glycine	2	64.4	< 0.001	4	152.1	< 0.001	8	68.3	< 0.001
Glutamic acid	3	61.5	< 0.001	2.5	121.3	< 0.001	7.5	50.1	< 0.001
Proline	-	-	-	4	4.0	0.020	-	-	-
N_{mic}	5	974.7	< 0.001	4	433.2	< 0.001	20	100.7	< 0.001

701 Note: Total free amino acids (FAA); dissolved organic N (DON); microbial biomass-N (N_{mic}).
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703